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Microbial degradation of electronic insulation polyimides has been evaluated using electrochemical impedance spectroscopy (EIS). Degradation of polyimide films was observed during incubation, showing two steps of development. Penetration of water and solutes into the polymer matrix was followed by deterioration of the polymer. The three fungi isolated and identified, Aspergillus versicolor, Cladosporidium cladosporidae, and a Chaetomium species, are commonly found environmental contaminants. Our results suggest that polvimides used in the electronic industry are susceptible to microbial degradation.

We have tested susceptibility of composites to a fungal consortium isolated from degrading polymers. All materials tested were found to be susceptible to the fungal attack. Penetration of composite resins by the fungi was observed. Reinforcing carbon and glass fibers were readily colonized. Data showing fungal growth on composite extracts provided evidence that organic constituents of composite materials serve as a source of carbon and energy for microbial growth. Our results indicate that composite materials are susceptible to attack by common air-borne fungal species.

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FINAL REPORT

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Grant — Interaction Between Microorganisms and Polymers Used For Layering In the Electronic Industry

Grant Number — F49620-92-J-0108

Principal Investigator — Professor Ralph Mitchell
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October 1995

1. ABSTRACT

Microbial degradation of electronic insulation polyimides has been evaluated using electrochemical impedance spectroscopy (EIS). A fungal consortium isolated from degrading polyimides, was used in this study. Degradation of polyimide films was observed during incubation, showing two steps of development. The Bode magnitude, the phase angle, and the Nyquist plots were all in good agreement with the data obtained. The first reaction resulted in penetration of water and solutes into the polymer matrix during the early stage of polymer-water contact. This was followed by the deterioration of the polymer, indicated by a large decrease of impedance in the Bode magnitude, progressive bending in the phase angle, and the appearance and compression of the semicircles in the Nyquist plots. These reactions strongly suggest polymer degradation and delamination. However, they were not observed with sterile control EIS cells. The three fungi isolated and identified, *Aspergillus versicolor*, *Cladosporidium cladosporidae*, and a *Chaetomium* species, are commonly found environmental contaminants. Our results suggest that polyimides used in the electronic industry are susceptible to microbial degradation.

We tested the microbial colonization and susceptibility of five composites to a fungal consortium isolated from degrading polymers. All materials were found to be susceptible to the colonization of the fungi, particularly a composite containing fluorinated polyimides and glass fibers. Penetration of composite resins by the fungi was also observed. Reinforcing carbon and glass fibers were readily colonized by the fungi. Data showing fungal growth on composite extracts provided evidence that organic constituents of composite materials serve as a source of carbon and energy for microbial growth. Our results indicate that composite materials are susceptible to attack by common air-borne fungal species.

2. OBJECTIVES

This project had two objectives. The first was to investigate the microbial degradation of electronic insulation polyimides using electrochemical impedance spectroscopy (EIS). Polyimides were chosen because they are important electronic insulators in layering of electronics. They are also widely used in fiber-reinforced polymeric composites (FRPC's) that are resistant to high temperature conditions. The second objective of this study was to determine the microbial susceptibility of FRPC's used in aviation.

3. ELECTRONIC INSULATION POLYIMIDES

3.1. Experimental

Electrochemical impedance spectroscopy (EIS). EIS cells were constructed by glueing a round piece of Kapton polyimide (pyromellitic dianhydride and 4, 4'-diaminodiphenyl ether) film (E. I. Du Pont Co., Wilmington, Delaware) onto a 316 stainless steel coupon (50.0 X 50.0 mm) by a conductive silver epoxy (SPI Instrumental, West Chester, Pennsylvania). On the polyimide film, a 30.0 mm long acrylic tube (I.D., 34.9 mm; O.D. 38.1 mm) was attached to the polymer-stainless steel coupon by a mixture of Amercoat 90 resin (Ameron, Protective Coatings Group, Brea, California) and Epon 828 resin (Shell Chemical Co., Houston, Texas) in a ratio of 4:1. A schematic diagram of the EIS cell used in our study is shown in Figure 3.1. After curing, the internal and external surfaces of the constructed EIS cells were thoroughly sterilized with 70% ethanol and left to dry in a laminar-flow sterile hood.

Our EIS consists of a Schlumberger 1250 frequency response analyzer combined with a Schlumberger 1286 electrochemical interface (Schlumberger Technologies - Instruments Division, Billerica, Massachusetts). Z-plot software (Scribner Associates, Inc., Charlottesville, Virginia) was used to manipulate the system. During data acquisition, samples were potentiostatically held at their open circuit potential (OCP), and a sinusoidal perturbation of 20-50 mV was applied to the system. The impedance response was measured over a range of frequencies from 65 kHz to 1 mHz and spectra were recorded as a function of immersion time at ambient temperature and pressure. OCP's were monitored versus a saturated calomel electrode as a reference electrode of the trielectrode system. Platinum mesh was used in the EIS cell as a counter electrode, and the EIS cell as a working electrode. In all experiments, surface areas of the working

electrode were 38.3 cm^2. Both Bode magnitude and phase angle plots as well as the Nyquist complex plane plots were used to provide information on increases in porosity, local defects and delamination.

Initially, a volume of 15.0 mL of sterile 0.2 *M* NaCl solution was added into the acrylic tube of the working electrode, and followed by 1.0 mL of a minimum salt solution. The salt solution consisted of (g per liter): K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g; $CaSO_4 \bullet 2H_2O$, 0.05 g; $MgSO_4 \bullet 7H_2O$, 0.5 g; $FeSO_4 \bullet 7H_2O$, 0.01 g; and $(NH_4)_2SO_4$, 1.0 g. The measurement of the impedance responses was made after equilibration of the system. The uniformity of all prepared EIS cells was evaluated to determine the validity of using them in subsequent monitoring and to assign them to different treatments. The EIS cells used as working electrodes were divided into two groups. One set of the prepared EIS cells (four) were inoculated with 100 μL of a fungal consortium, that was maintained on a malt extract medium (Difco Lab., Detroit, Michigan). The consortium was obtained by an enrichment process on degraded polyimides. Another set of EIS cells was kept sterile throughout the study by addition of 100 μL 0.1% sodium azide in the 0.2 *M* NaCl solution. At weekly or biweekly intervals, all EIS cells were analyzed for their impedance responses. The tri-electrode system was housed in a sterile laminar-flow hood. Aseptic procedures were used throughout to avoid contamination and cross contamination of the EIS cells. Both impedance and phase angle plots as well as Nyquist complex plane plots were used in the interpretation of polyimide performance. At the end of study the polymer film from inoculated and sterile EIS cells were taken and prepared for examination by scanning electron microscopy (SEM).

Scanning electron microscopy (SEM) sample preparation. Polyimide film samples from the inoculated and the sterile control EIS cells were treated with 3% glutaraldehyde buffered with 0.2 *M* sodium cacodylate overnight. The solution was previously filtered through a 0.2-μm-pore-size polycarbonate membrane filter (Gelman Science, Ann Arbor, MI). Film samples were then washed with 0.2 *M* Na cacodylate three times, fixed in 1% osmium tetroxide with 0.1 *M* Na cacodylate, and rinsed with 0.2 *M* Na cacodylate and deionized water three times for each treatment. The samples were dehydrated by immersing in an ethanol-distilled water series of 40, 60 70, 80, 85, 90, 95, and 100% ethanol. Samples were stored in 100% ethanol and air-tight sealed glass vials before

being critical point dried in liquid CO_2 (Smdri PVT-3B, Tousimis Research Co., Rockville, MD). Following drying, they were immediately coated with gold-palladium and viewed under an AMR 1000 Scanning Electron Microscope.

3.2. Results

Degradation of polyimides was monitored in inoculated and sterile EIS cells containing a 0.2 *M* NaCl solution for 122 days of incubation under ambient conditions. Polyimides were fixed on a conductive stainless steel coupon to enhance current transfer crossing the polymer barrier (Fig. 3.1). A hypothetical presentation of polymer performance is presented in Fig 3.2. No apparent difference in electrochemical property between the inoculated and the sterile cells was observed at the initiation of the experiment, indicating the uniformity and excellent resistivity of the polyimide films (Fig. 3.3a and 3.4a). However, a decrease of impedance in the lower frequency region (10 to 10^{-2} Hz) was detected in the inoculated EIS cells after 17 days and in the sterile cells after 24 days. This initial decrease of pore resistance in both the inoculated and the sterile EIS cells was due to the adsorption of moisture and ionic species into the polymer matrix resulting in a decrease of the film resistivity. Inoculation of a fungal consortium showed enhancement of this transportation process, probably due to fungal activity and degradation of the polyimides. However, this process was not as clearly identified in the Bode phase angle plots (Fig. 3.3b and 3.4b) as in the Bode magnitude plots (Fig. 3.3a and 3.4a), suggesting no degradation of the polyimides. The appearance of a second time constant in the phase angle plots is a direct indication of the coating polymer degradation.

The second decline of impedance, observed in the inoculated cells but not the sterile control cells (Fig. 3.3a and 3.4a), signified a further decrease in capacitance of the polymer film. Impedance magnitude was decreased from 10^8 at time zero to below 10^5 in the inoculated EIS cells within 72 days. During the same time period of incubation, sterile controls did not show similar spectra. This second change also reflected in the Bode phase angle plots, in which a second time constant was resolved, indicated the existence of at least one localized pore in the polymer. The presence of only one time constant in the sterile cells over the whole period of incubation supported the fact that the polymer was still intact after exposure to the sterile medium. A high impedance value indicates the intact non-porous nature of a coating.

The Nyquist complex plane plots of the inoculated EIS cells clearly differed from the sterile EIS cells in that the semicircles were observed over the time of incubation in the former, specifically after 72 days (Fig. 3.5). The appearance of the semicircles coincided with the second time constant observed in the Bode phase angle plot, and also the second decline of impedance in the Bode magnitude plots of the inoculated cells. However, such results were not observed with our sterile EIS cells. Data from this study also indicated the degree of semicircle compression between 72 days and 122 days, suggesting the relationship between the semicircle compression and the severity of deterioration of the polymers.

The relationship between the decrease of pore resistance and the polymer degradation in the presence of fungi was confirmed by drilling a series of needle sized small holes in the polymeric film that had previously exposed to sterile conditions for over 122 days and proven to be intact from its EIS spectra (Fig. 3.6). After each hole was drilled, electrochemical data of the damaged EIS cell were collected and analyzed for Bode magnitude and phase angle changes. When one needle-sized hole was created in the polymer, pore resistance decreased drastically. In response to an increased number of holes in the polymer, impedance spectra showed successive decreases, the difference between the intact and damaged film was much greater than that between the films with different numbers of holes in it. The effect of artificial pores in the polymer was also demonstrated in the phase angle plots in which a second time constant was resolved in the saddle shaped curves, indicating severe damage of the polymer film on metal surfaces. The damage to the polymer by drilling also was clear in the Nyquist complex plane plots where the appearance and compression of the semicircles could be seen only after drilling. As the number of holes in the polymer increased, the radius of a semicircle tended to decrease (Fig. 3.6). Because of the physical nature of penetration, phase angle plots before and after drilling deviated greatly due to the drastically change in the polymer characteristics. This information offers insights into the nature and extent of the film biodeterioration after exposure to a fungal consortium. As expected, the degree of damage by fungal activity was less severe than that by drilling; but the continuous deterioration by fungi may result in devastating consequences when the polymers are used as electronic insulators.

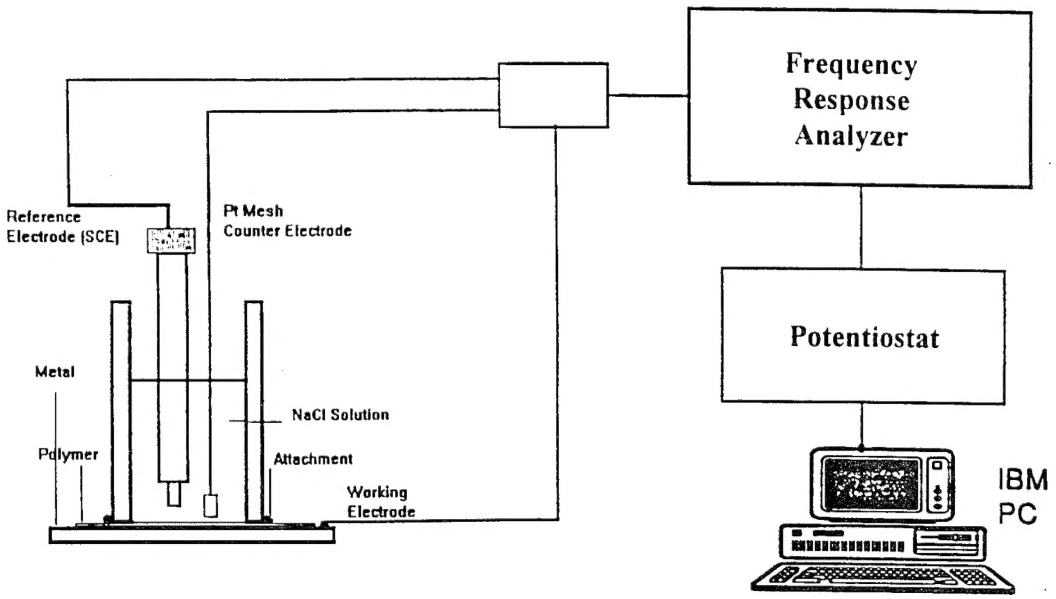
EIS measures the resistive, capacitive, and inductive components of the overall interfacial impedance. The polyimide film acts as a resistor in the high frequency region, while it acts as a capacitor in the low frequency region. Therefore, it is clear that the film resistivity was fundamentally changed after 72 days exposure to the fungal consortium, because the impedance of an organic polymer on a conductive surface depends primarily on the transport of ionic species across the material matrix. Furthermore, EIS proves to be a very sensitive technique for the detection and prediction of coating failure. As we observed in the inoculated and the sterile EIS cells, the deterioration processes at the polymer-metal interface can be distinguished with appropriate experimental design. EIS is particularly useful for studying non-conducting and semi-conducting thin films and is, therefore, ideally suited to the monitoring of organic coatings. Coating deterioration (increased permeability) and increased corrosion reactions underneath coatings are reflected in the impedance spectra. The degree of coating delamination can be resolved in the Bode and Nyquist plots. We have also applied EIS to the study of biodeterioiration of protective coatings and fiber reinforced polymeric composites. This study is described in section 4 of this report.

At the termination of our EIS incubations, polyimide films from the inoculated and sterile EIS cells exposed to a fungal consortium for 122 days were examined by scanning electron microscopy (SEM). Two SEM micrographs showing fungal colonization of the surface of polyimides from the inoculated cells are presented (Fig. 3.7). In contrast, two SEM micrographs of the sterile EIS cell polymer show no fungal colonization (Fig. 3.8). Fungi were responsible for the deterioration of coating electrochemical properties as demonstrated by EIS. The species of fungi, *Aspergillus versicolor*, *Cladosporidium cladosporidae*, and *Chaetomium* spp., were isolated and identified from a sample of the polyimide. All of these fungi are ubiquitous in natural environments.

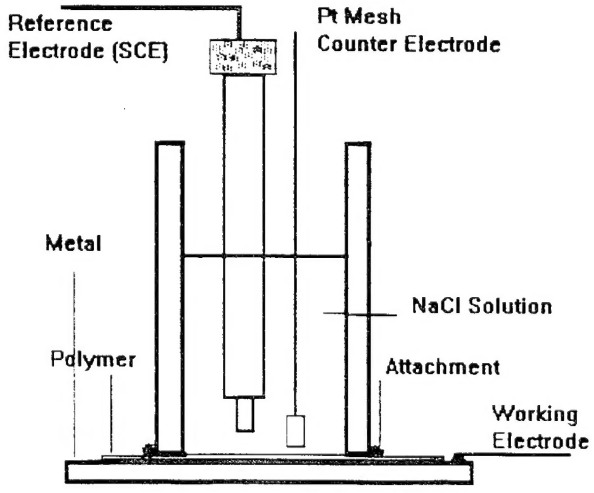
3.3 Conclusions

Microbial penetration and degradation of polyimides has been detected using impedance spectroscopy. Our data support the hypothesis that electronic packaging polyimides are susceptible to attack by common air-borne fungal species. Research in our laboratory has focused on the elucidation of the biochemical mechanisms involved during fungal degradation of these polymers.

Fig. 3.1 Schematic illustration of (*a*) the electrochemical impedance system used, and (*b*) an electrochemical cell containing a polyimide film



a



b

Fig. 3.2 Theoretical presentation of the (*a*) Bode magnitude and (*b*) phase angle, and the (*c*) Nyquist plots of a deteriorating polymer or coating

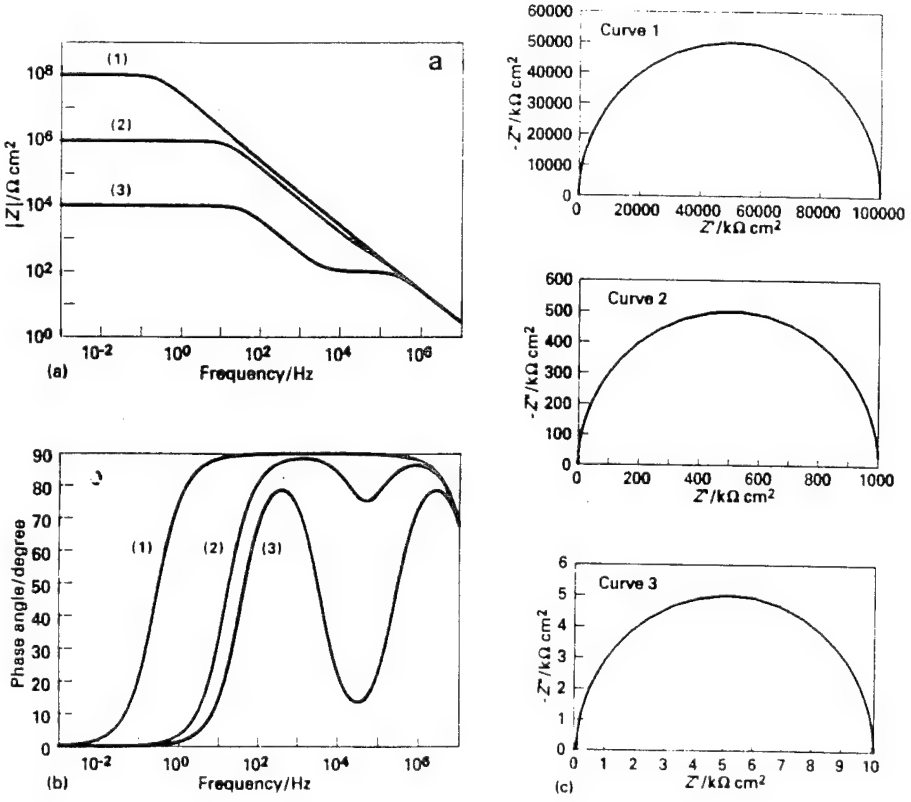


Fig. 3.3 (*a*) Bode magnitude and (*b*) phase angle plots of polyimides inoculated with a fungal consortium and incubated at ambient conditions

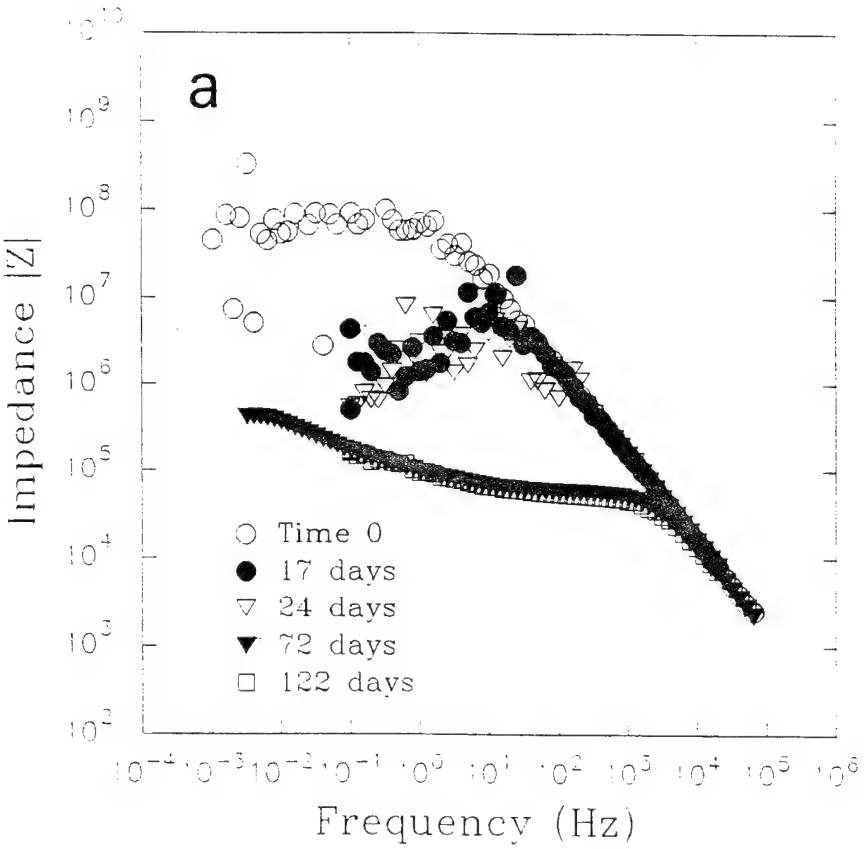


Fig. 3.3 Continued

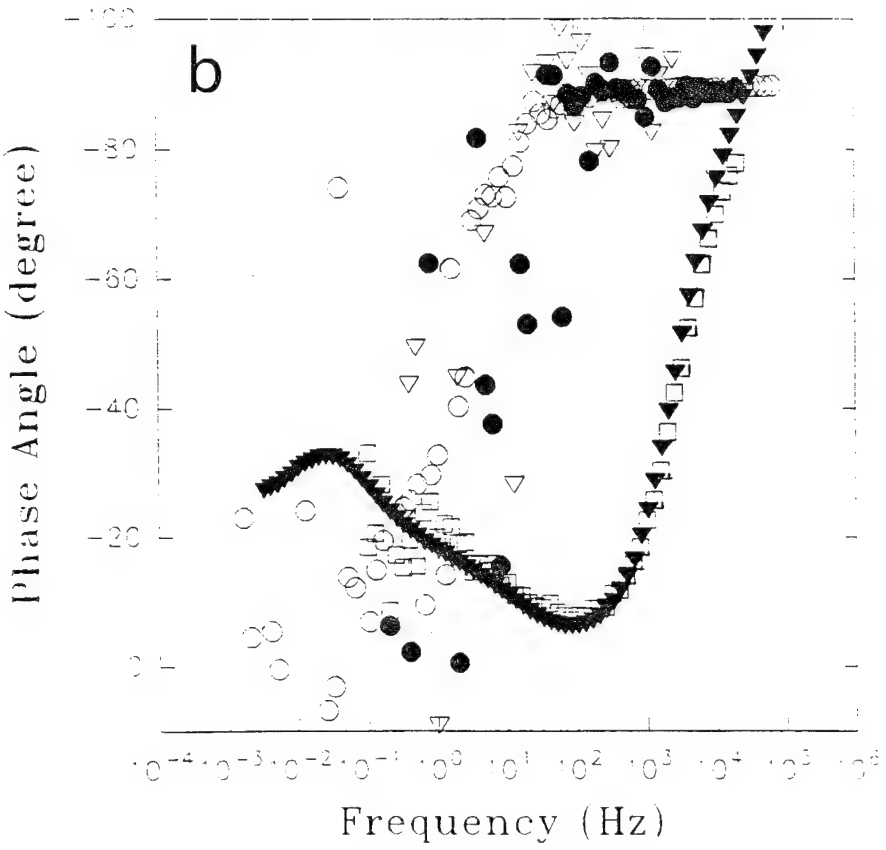


Fig. 3.4 (*a*) Bode magnitude and (*b*) phase angle plots of polyimides held under identical condition as Fig. 1.3 except for the fungal inoculation

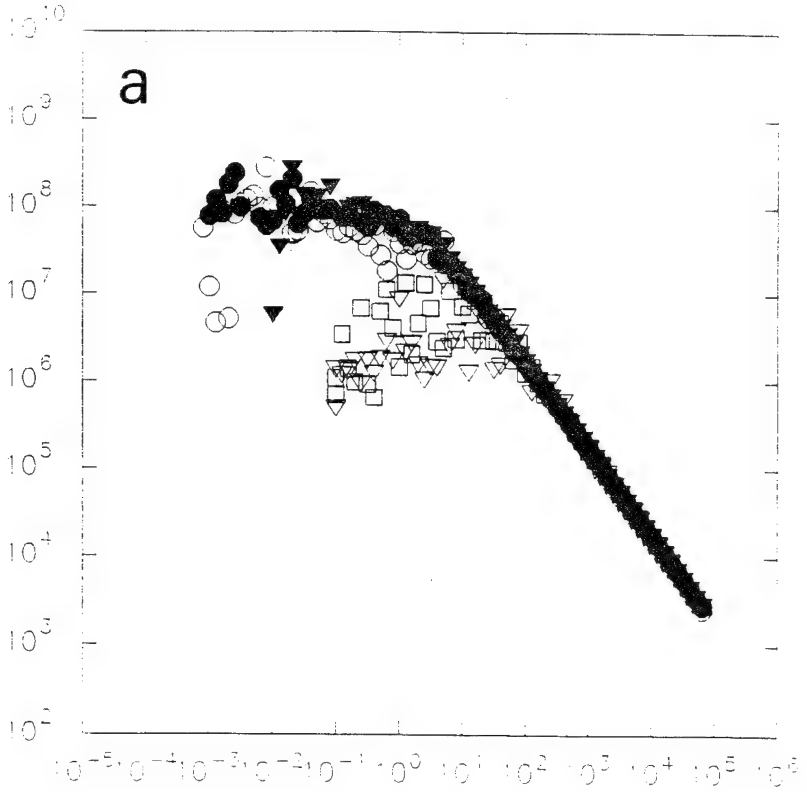


Fig. 3.4 Continued

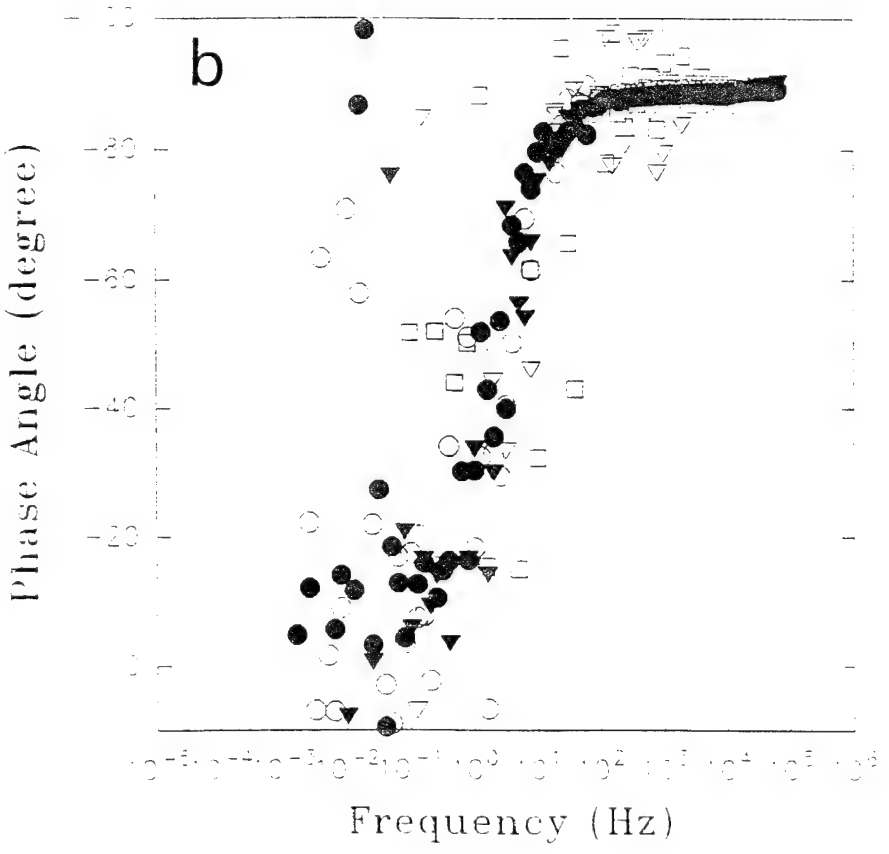


Fig. 3.5 Nyquist complex plane plots of (*a*) the inoculated and (*b*) the sterile EIS cells

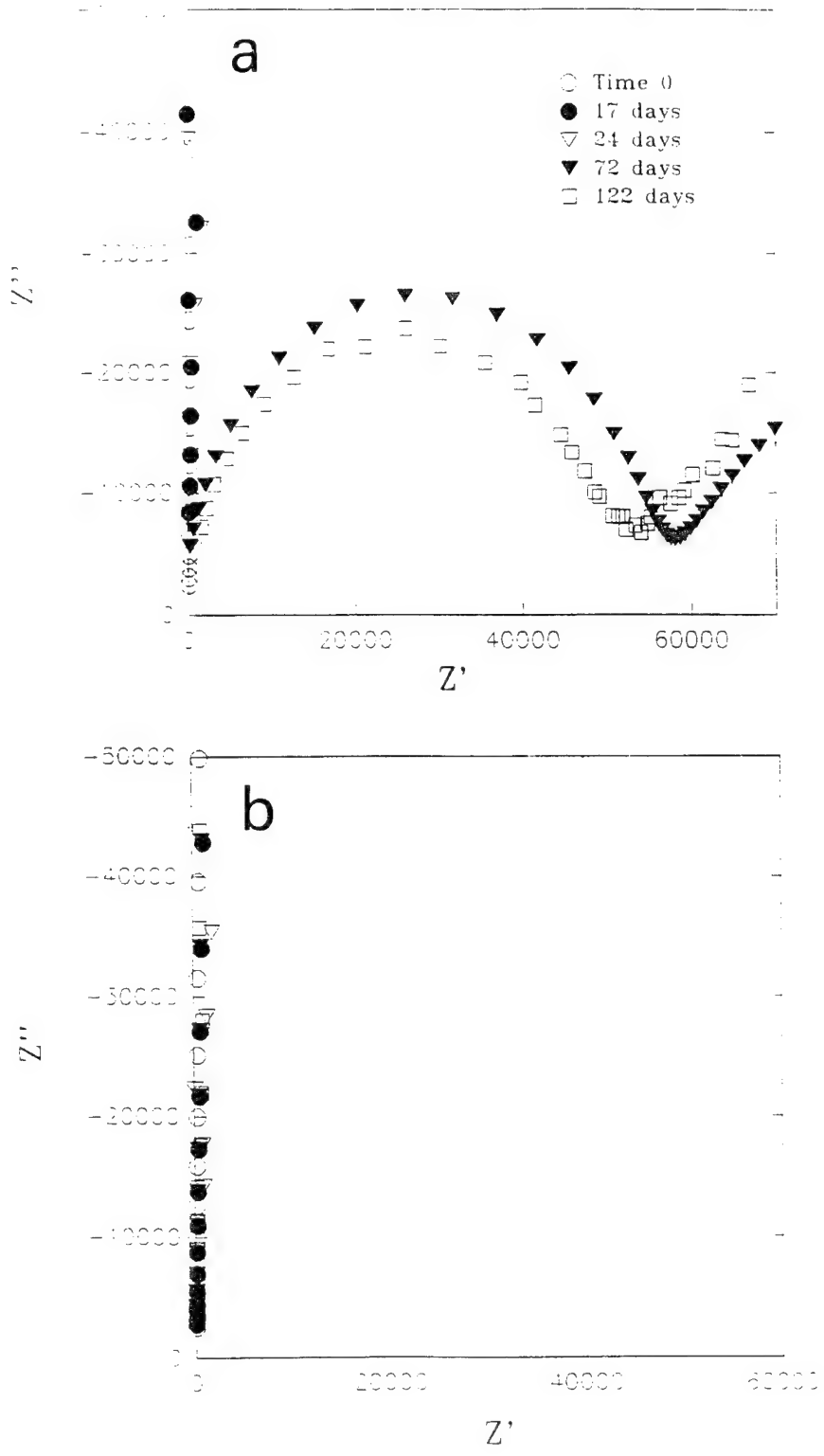


Fig. 3.6 Nyquist complex plane plot of the sterile EIS cell that was intact after exposure for 122 days under sterile conditions and subsequently drilled with three needle-size holes

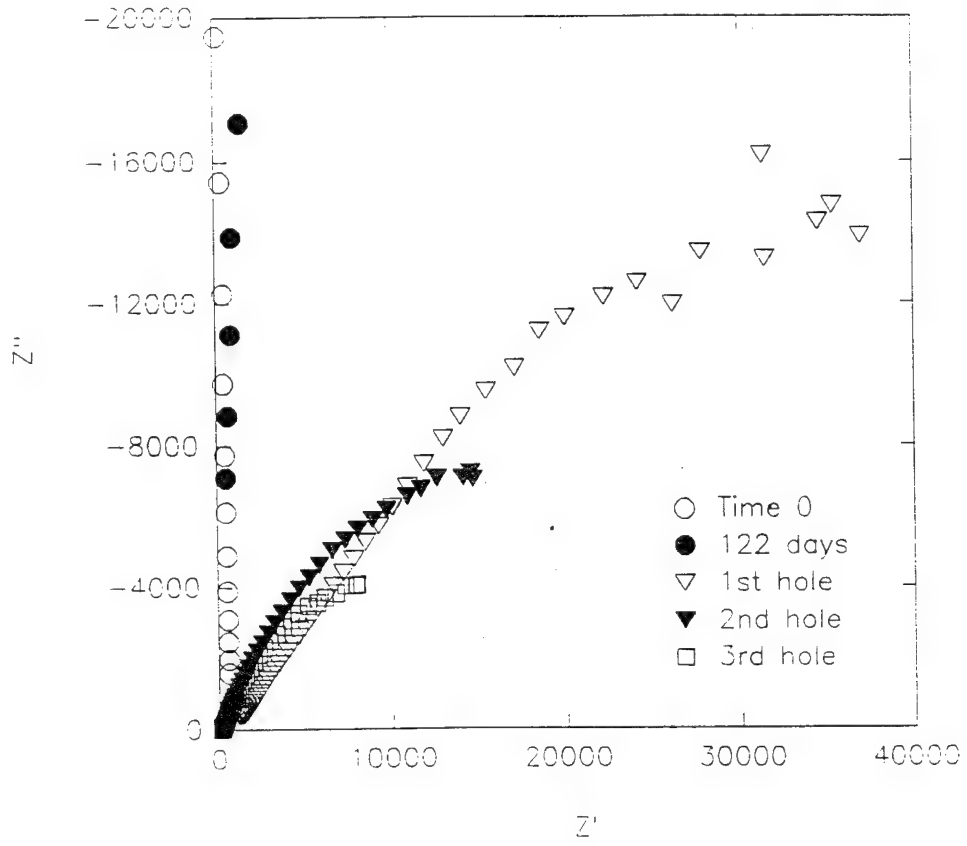


Fig. 3.7 SEM micrographs of biodeteriorated polyimides from the inoculated EIS cell showing fungi on the polyimide film

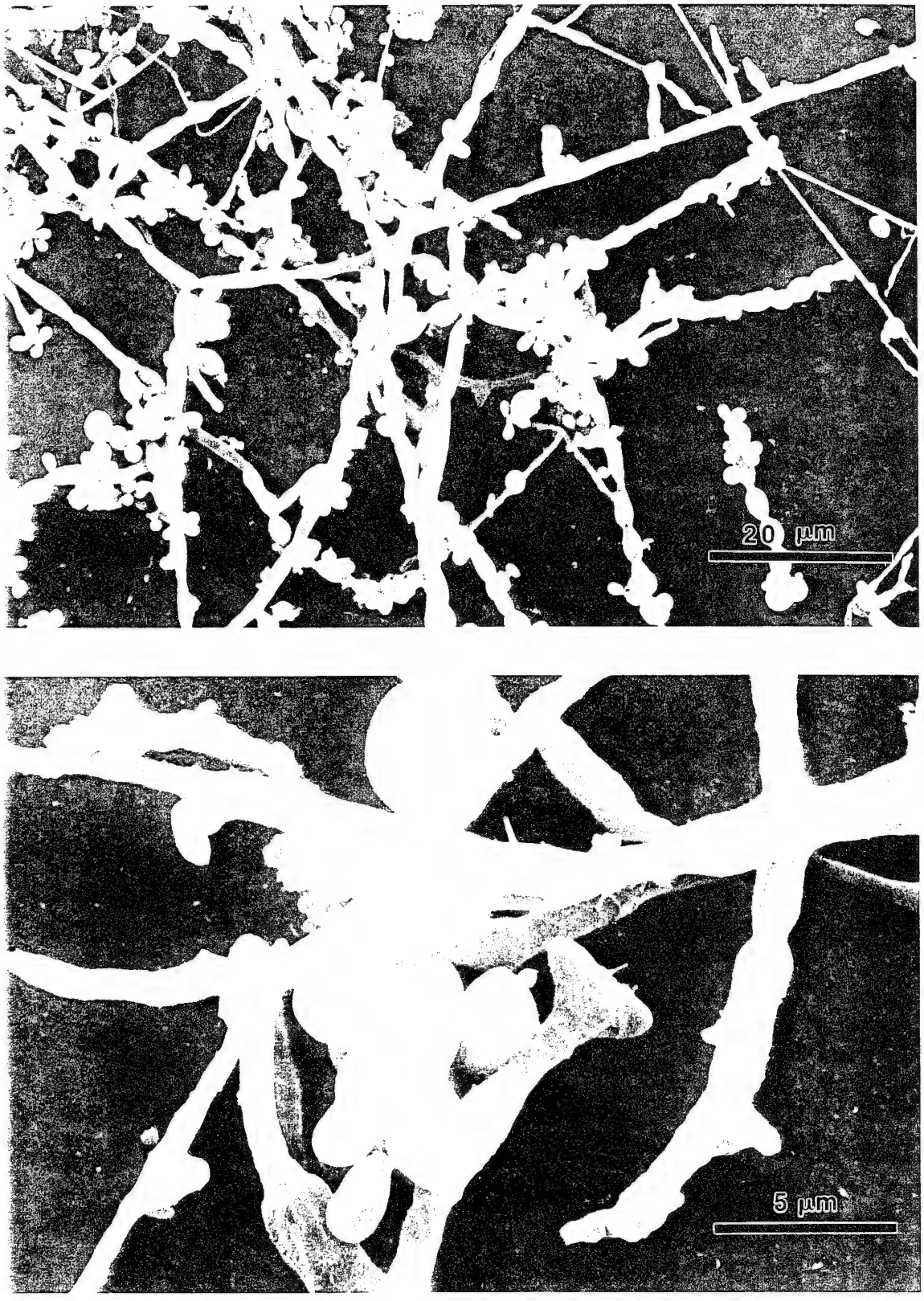
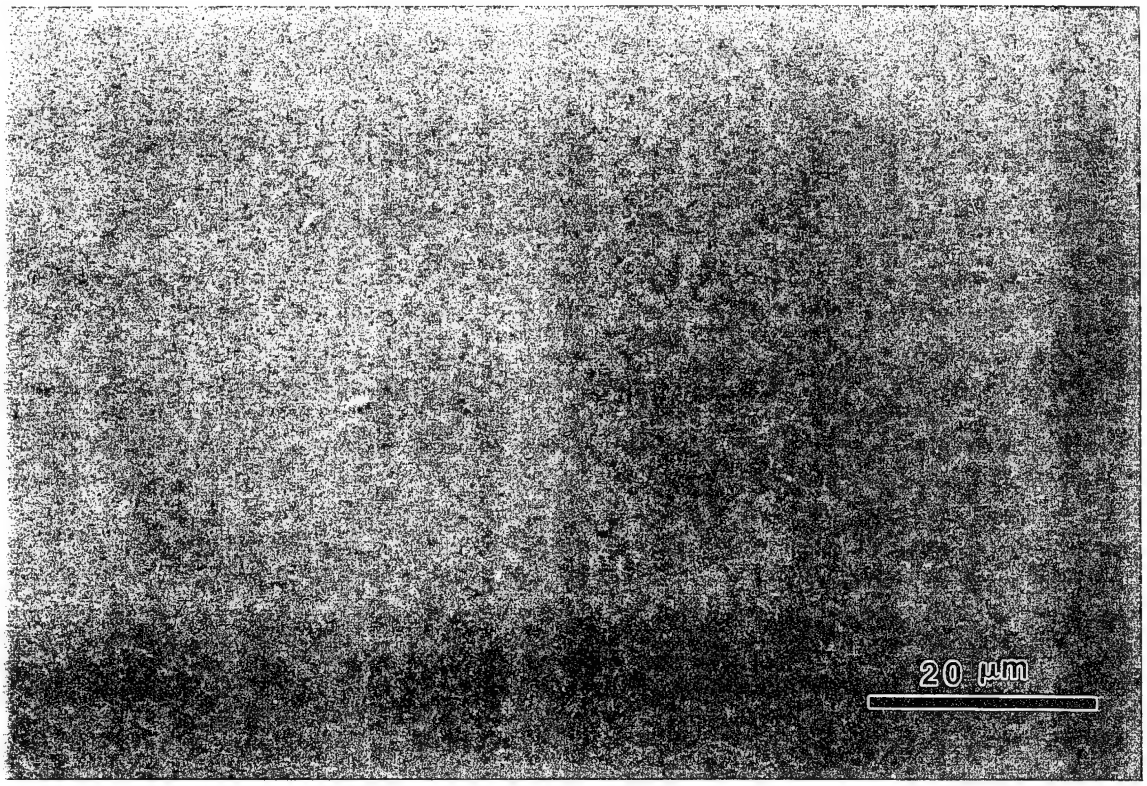
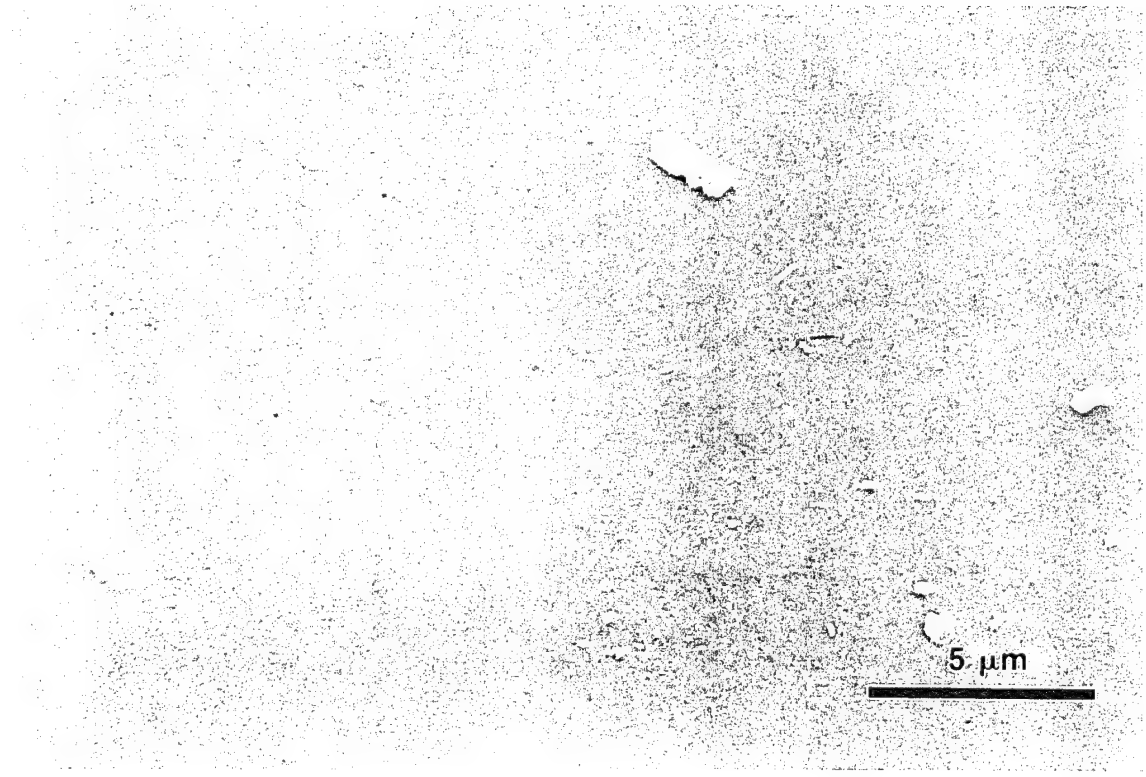


Fig. 3.8 SEM micrographs of intact polyimides from the sterile EIS cell after 122 days of incubation





4. FIBER REINFORCED POLYMERIC COMPOSITES

4.1. Experimental

Incubation studies. Composite materials (labeled from A to E) were provided by the University of Dayton Research Institute, Dayton, Ohio. The composition of the materials tested is shown in Table 4.1. They were cut into 10 x 20 mm coupons which were sterilized by immersing in 70% ethanol and dried in a laminar flow hood. The coupons were suspended in flasks containing malt extract broth (Difico Lab., Detroit, Michigan), consisting of (g L^{-1}) malt extract, 6.0; maltose, 1.8; glucose, 6.0; and yeast extract, 1.2. The medium was inoculated with a fungal consortium isolated from FRPC's and identified as *Aspergillus versicolor*, *Cladosporium cladosporioides*, and a *Chaetomium* spp.. Incubation was on a shaker in the dark at room temperature (22 $\pm$ 2°C). Samples were taken at regular intervals and prepared for scanning electron microscopy (SEM).

We tested commercial fibers P-25 and P-100 (Amoco Performance Products, Inc., Greensville, South Carolina), MJ-60 (Toray Industries, Tokyo), and Fisherbrand glass fibers (Fisher Scientific, Pittsburgh, Pennsylvania). They were cut into 30 mm bundles and autoclaved before introduction into the culture flasks containing malt extract broth described above. At monthly intervals, fiber samples were prepared for examination by scanning electron microscopy (SEM).

Growth on composite extracts. Two coupons (10 x 20 mm) from each composite were autoclaved in flasks containing 80 mL of a minimum salt medium for 20 min. The salt medium consisted of (g L^{-1}): K_2HPO_4 0.8, KH_2PO_4 0.2, $CaSO_4 \bullet 2H_2O$ 0.05, $MgSO_4 \bullet 7H_2O$ 0.5, $FeSO_4 \bullet 7H_2O$ 0.01, and $(NH_4)_2SO_4$ 1.0. When cool, the coupons were removed and the composite extracts were inoculated with 100 µL of the fungal consortium. All flasks were incubated at 22°C and ambient pressure in the dark. After one month incubation, aliquots was analyzed for turbidity at 600 nm.

SEM sample preparation: Samples of composite and fiber material were prepared for SEM examination as described in the polyimide section of this report.

4.2. Results

Five composite materials, provided by K. Thorp at the University of Dayton Research Institute, were examined for attack by fungi. All of the coupons were colonized by our fungal consortium after one month of incubation. SEM micrographs showed fungal colonization of composite A (Fig. 4.1). This material contains a fluorinated polyimide resin in addition to glass fibers. Additional SEM micrographs of the incubated coupons indicated that the other four composites were also susceptible to colonization by fungi (Fig. 4.2). Penetration of the composite A resin by fungal hyphae is shown in Fig. 4.3.

Microbial contamination of materials during the manufacturing process is a probable major contributor to the material degradation. Our microbial consortium used in their study was obtained from degrading polymeric materials and found to be active in the degradation of a range of polymeric materials.

When the autoclaved extracts of the composites used in our study were tested for utilization by fungi as the sole carbon and energy source, significantly higher growth was observed in extracts of all five composites (Fig. 4.4). The data support the hypothesis that chemical constituents of the composites serve as carbon and energy sources for the growth of fungi.

Our data showed that fungi have the ability to colonize fiber surfaces extensively. For example, 84 days after immersion the Amoco P-100 fibers were found to be completely coated with fungal hyphae (Fig. 4.5). In contrast control fibers from sterile flasks were coated with small deposits of chemical precipitates (Fig. 4.6). It is anticipated that sizing chemicals may be used by the fungi for growth similar to those observed with the composite extracts.

4.3 Conclusions

The five composites tested in this study were readily colonized by contaminating microorganisms, especially air-borne fungal species. Fungi were found to penetrate into the interior of the resin materials and utilize the organic chemicals for their growth, particularly with the fluorinated polyimide resin. Sizing chemicals on fibers may also contribute organic chemicals for the fungal growth. Our study suggests that microorganisms pose a serious threat to composite materials in environmental conditions where temperature and humidity are optimal for fungal growth, for example in warm humid climates.

Table 4.1 Chemical composition of composite materials used in this study

Composite	Resin	Fibers
A	Fluorinated polyimides	Glass
B	Bismaleimide	Carbon
C	Polyetheretherketone	Carbon
D[a]	Epoxy	Carbon
E[b]	Epoxy	Carbon

[a] Multidirectional orientation of fibers in the resins, [0, 45, 90, -45]2S

[b] Unidirectional orientation of fiber in the resins, [o]16T

Fig. 4.1 SEM micrographs of composite A colonized by a fungal consortium after 30 days of incubation at (*a*) low and (*b*) high magnifications

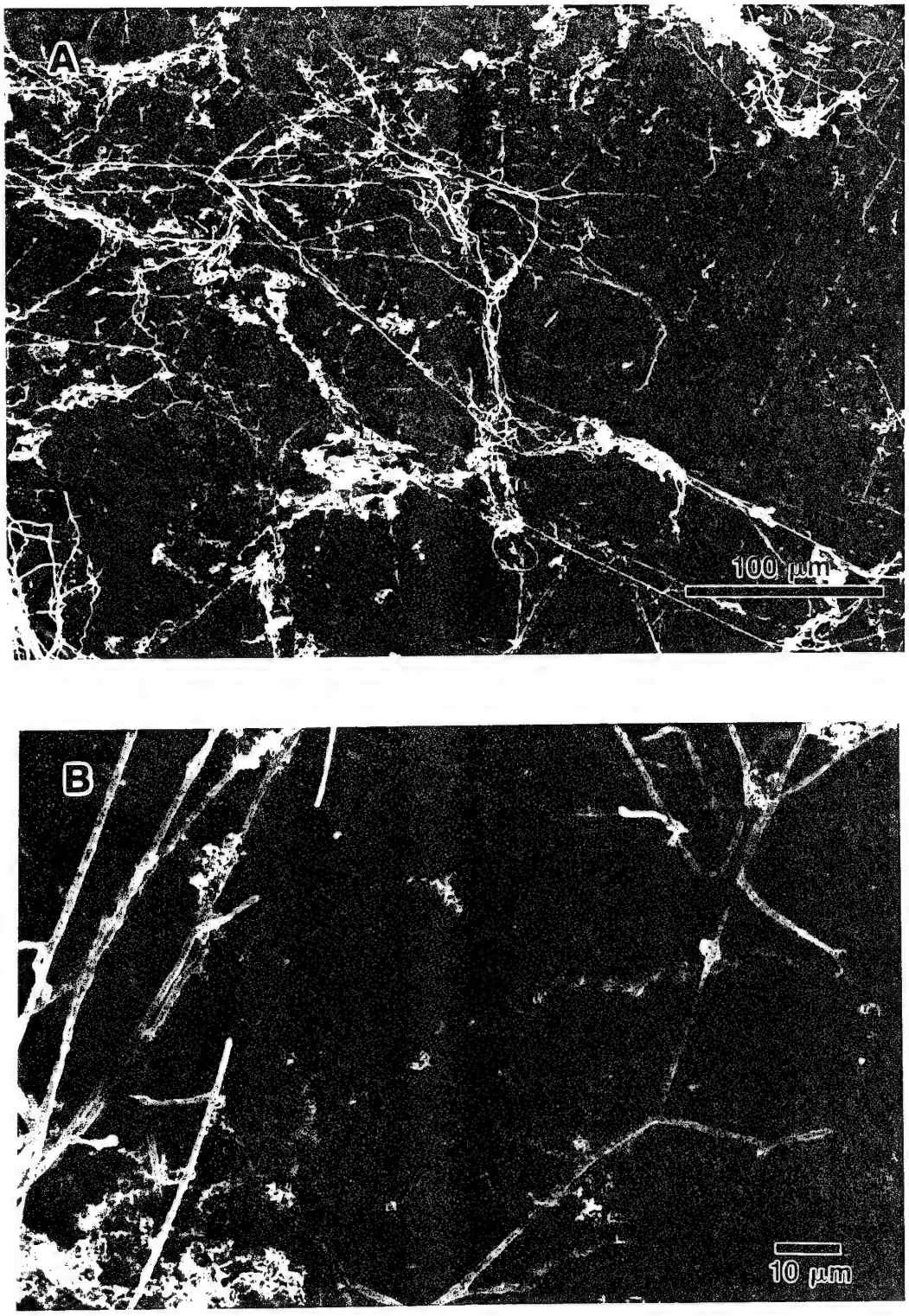
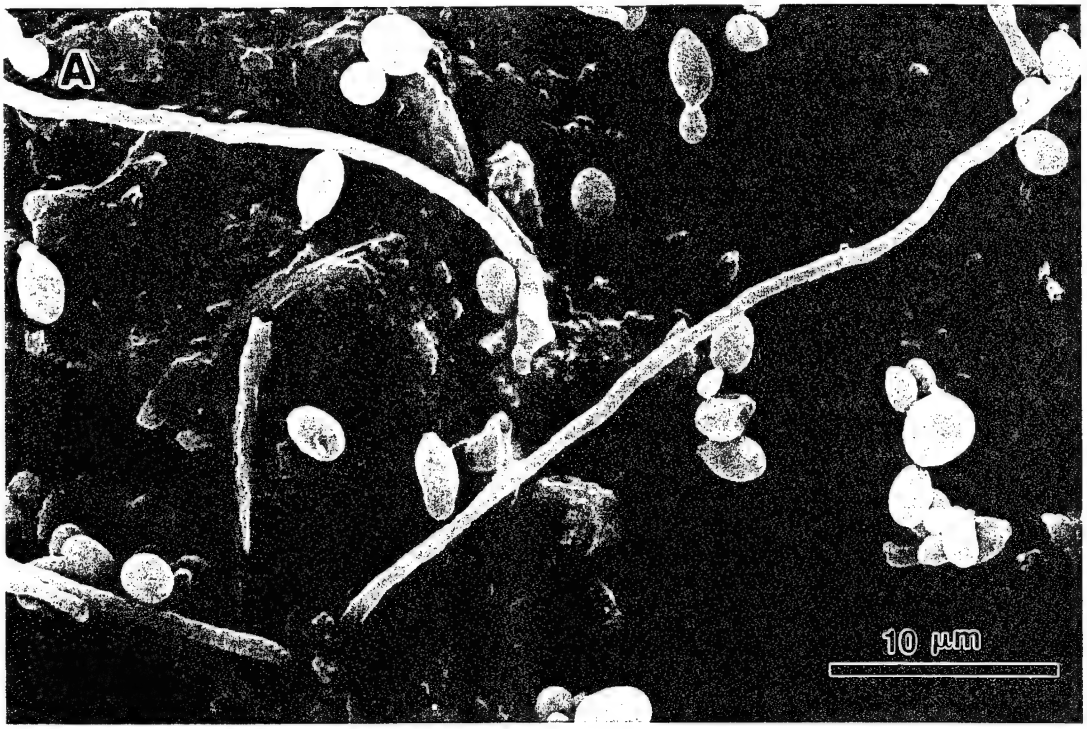


Fig. 4.2 SEM micrographs of composite B, C, D, and E colonized by fungi after 30 days of incubation. (*a*) Bismaleimide/carbon fibers; (*b*) polyetheretherketone/carbon fibers; (*c*) epoxy/multidirectional carbon fibers; and (*d*) epoxy/unidirectional carbon fibers



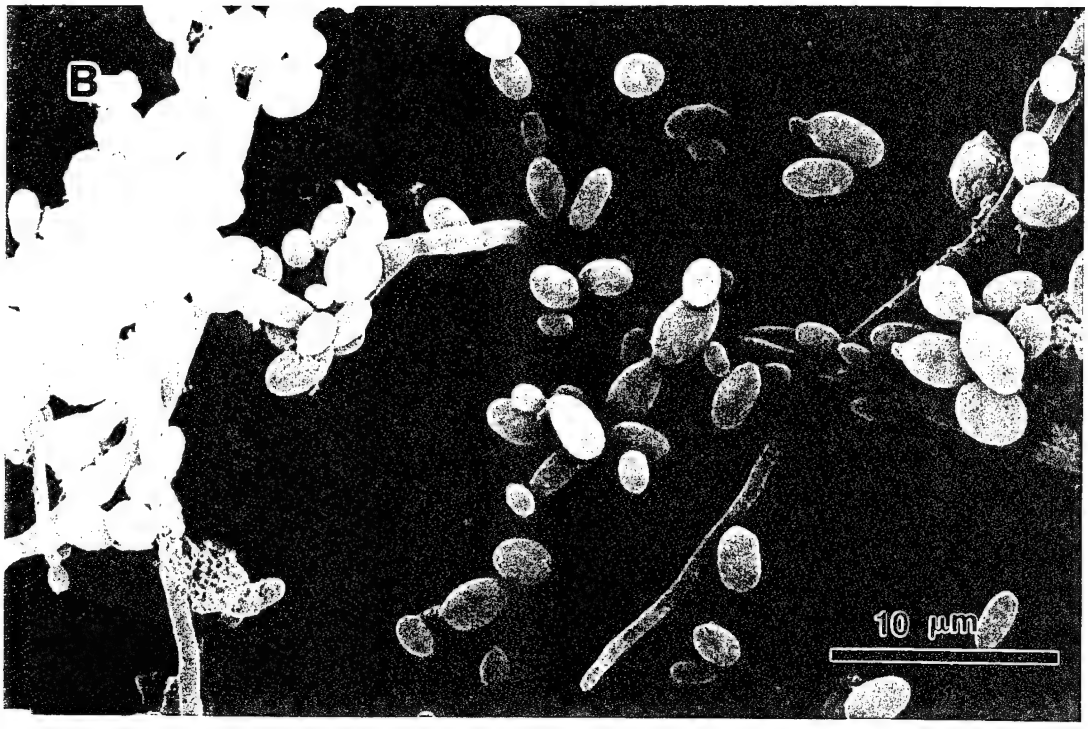


Fig. 4.2 Continued

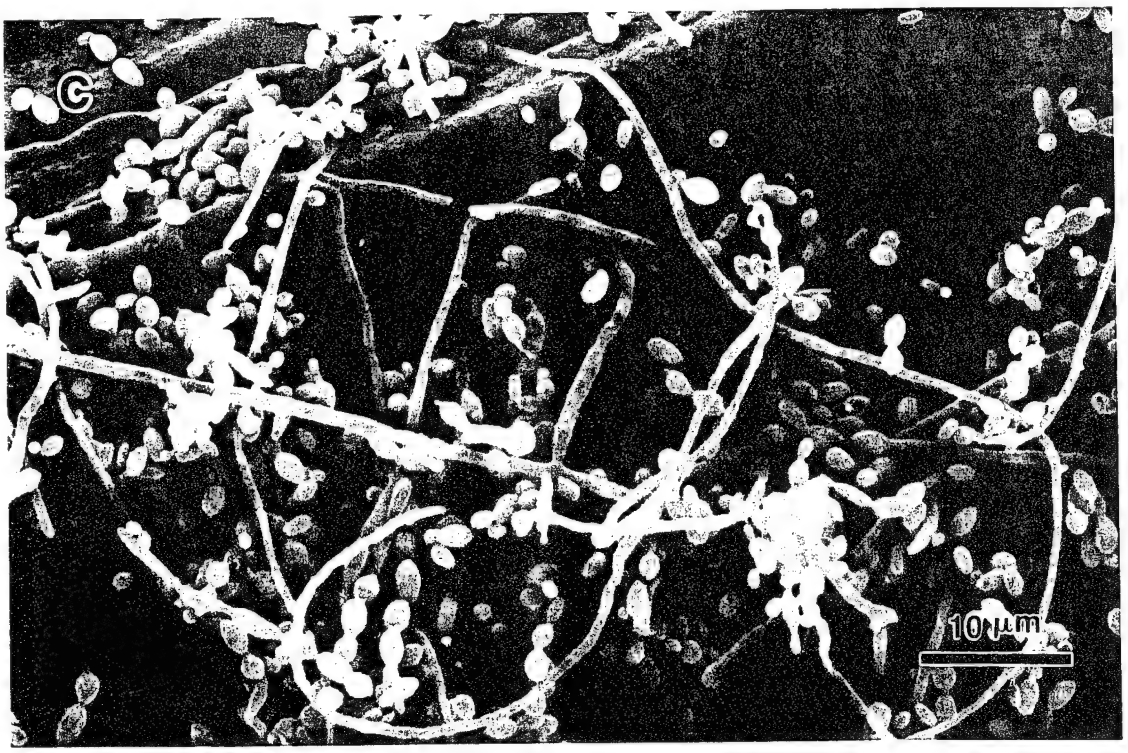


Fig. 4.3 SEM micrograph showing a close-up view of fungal penetration of composite A resin (indicated by an arrow) after 30 days of incubation

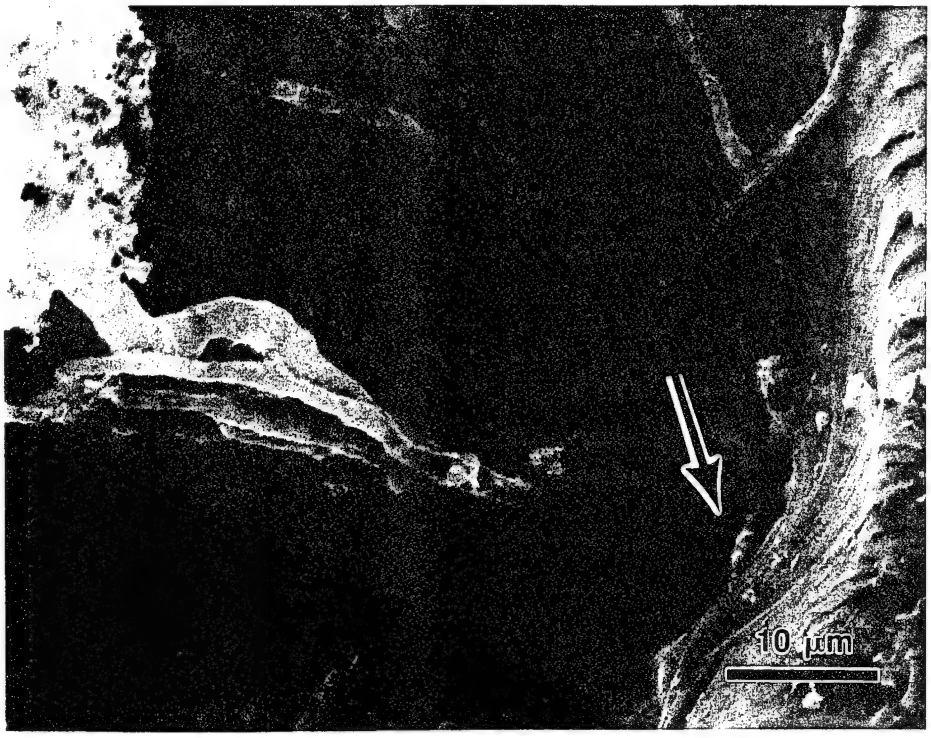


Fig. 4.4 Microbial growth on composite extracts as a source of carbon and energy after 30 days of incubation. (*1*) Fluorinated polyimides/carbon fibers; (*2*) bismaleimide/carbon fibers; (*3*) polyetheretherketone/carbon fibers; (*4*) epoxy/multidirectional carbon fibers; (*5*) epoxy/unidirectional carbon fibers; and (*6*) no composite control. Standard deviation represents means of three measurements.

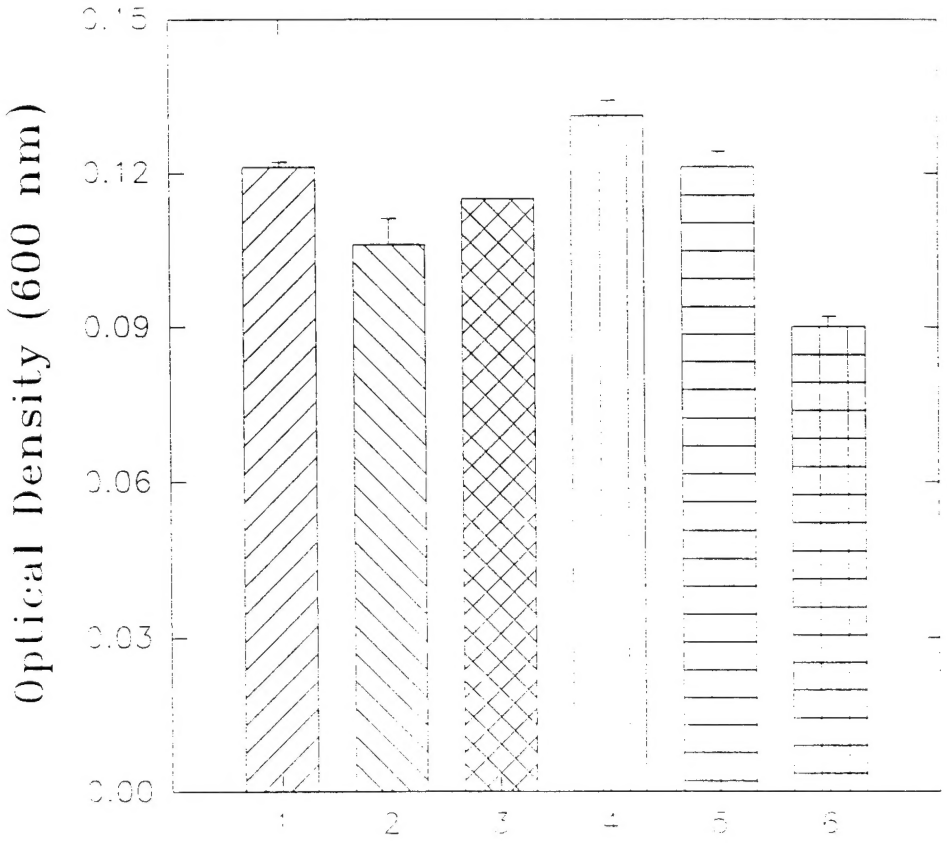


Fig. 4.5 SEM micrographs of a carbon fiber colonized by a fungal consortium after 84 days of incubation with a fungal consortium

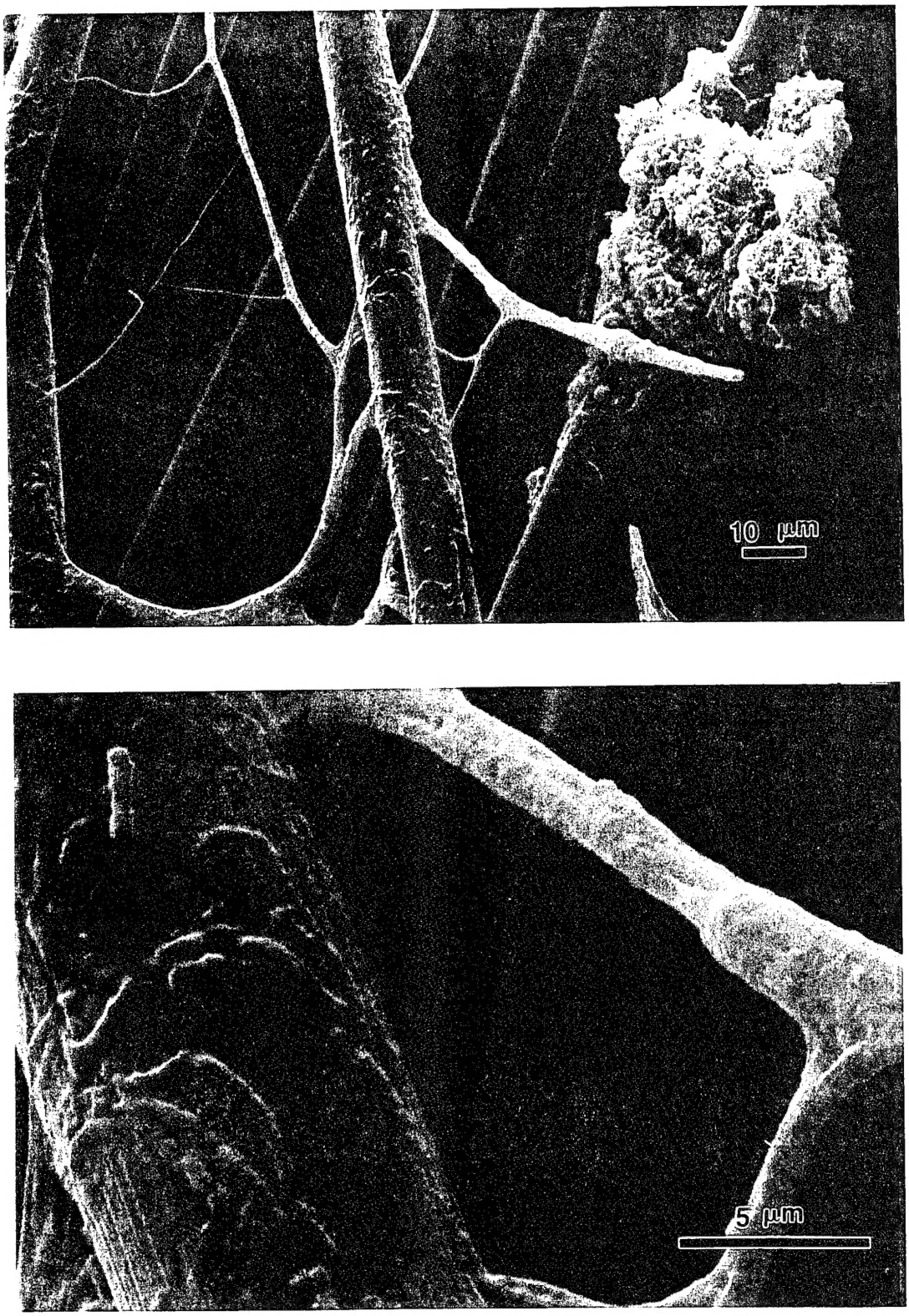
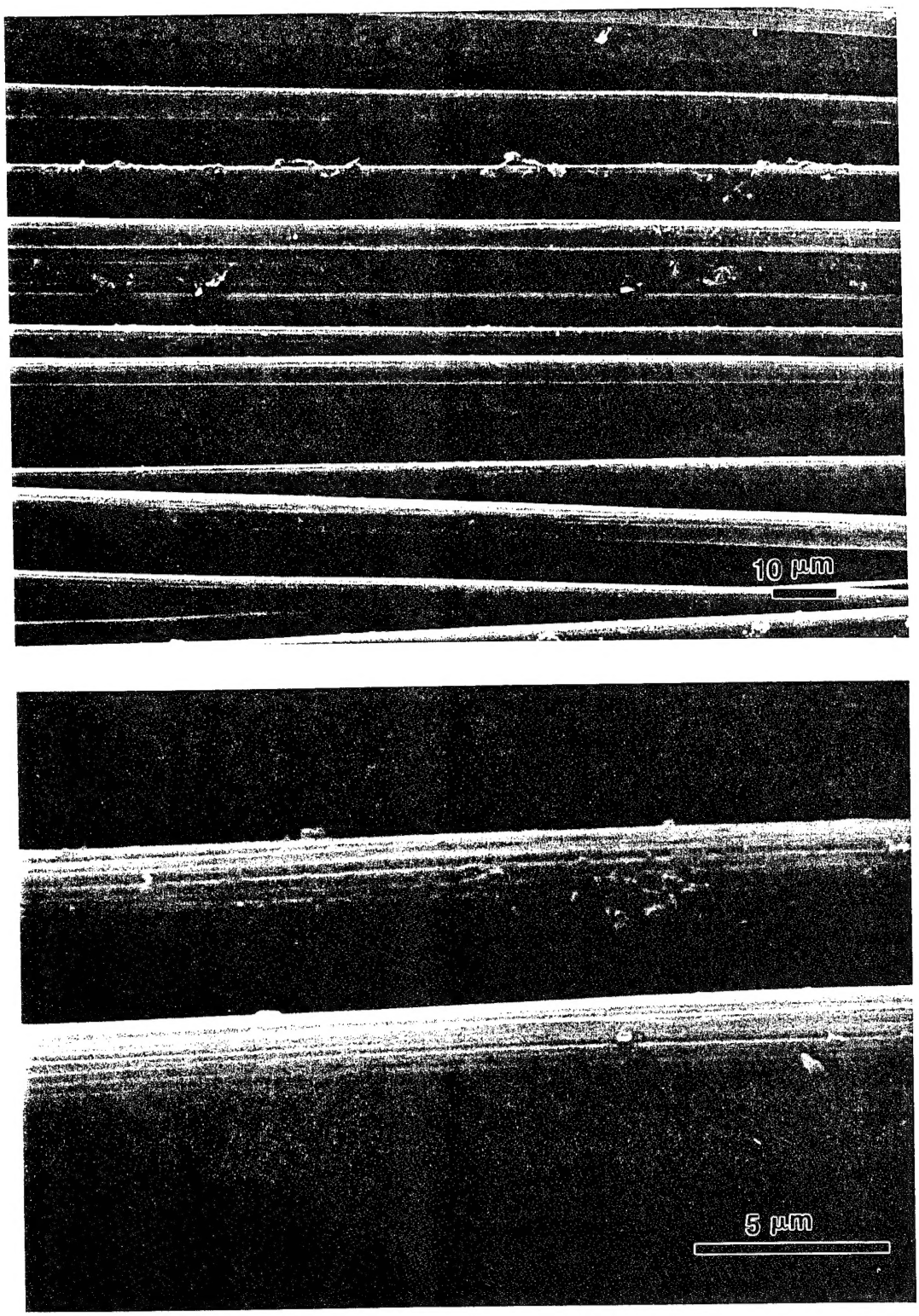


Fig. 4.6 SEM micrographs of a carbon fiber in sterile medium held for 84 days



5. PUBLICATIONS BASED ON RESEARCH SUPPORTED BY THIS GRANT

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6. LIST OF PEOPLE INVOLVED IN THIS PROJECT

1). Professor R. Mitchell

2). Professor T.E. Ford

2). Dr. Ji-Dong Gu